FINAL PERFORMANCE REPORT

IMMUNOTOXICOLOGY OF JP-8 JET FUEL: MECHANISMS

David T. Harris, J. Wang, D. Camacho, M. Badowski, T. Tsang and M. Witten
Dept. Immunobiology and Pediatrics, 1501 N. Campbell Ave, Rm 643 Life Sciences North, University of Arizona, Tucson, AZ 85724
Tel: (520) 626-5127; **FAX:** (520) 626-2100; **Email:** davidh@U.Arizona.edu

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Histopathology from In Vivo JP8 Exposures

C57BL6 mice were exposed to the standard 1000 mg/m^3 aerosolized JP8 jet fuel for 1h/day and assessed on days 1, 4 and 7 of exposure. The following assays were conducted in an effort to elucidate potential mechanisms of action of JP8 on the immune system: Immune organ weights and cell numbers, Cell viabilities, Histopathology, Cytokine production (by intracelluar FACS), Apoptosis (by 7-AAD/Annexin staining by FACS) and Hematopoietic progenitor cell assays (CFU). The following results were obtained.

Histopathology1. Liver, Lung, Cervical Lymph Nodes, Salivary Glands, Spleen and Thymus had no gross lesions/changes at either day 1, 4 or 7 of jet fuel exposure.
2. Kidney had slight lesions observed in all mice but only at day 4 of exposure.

HematologyDay 1: Monocytosis; Leukocytosis, Lymphocytosis, Eosinophilia in some mice; Polycythemia; high MCHC; SP mostly reversed
Day 4: Monocytosis & Lymphocytosis; Eosinophilia; high MCHC; Polycythemia; SP reversed some effects
Day 7: Neutrophilia; Polycythemia; high MCHC; Platelet number normal but low MPV; SP reversed some effects

[MCHC: indicates that hemolysis has occurred in vivo; could contribute to renal tubular nephrosis
Polycythemia: occurs when both RBC count & hemoglobin levels are elevated; may result from MDS (rare), hypoxia, secretion of EPO, or hydronephrosis
MPV: may be indicative of problems in platelet production]

Cytokines

1. Increased levels of inflammatory cytokine secretion from spleenic T cells were observed at days 1 and 4 of exposure (IFN-gamma, IL-10 and IL-4).

Hematopoietic Progenitor Cell Assays

1. There may be an effect on the bone marrow in terms of a temporary suppression followed by a rebound effect in terms of numbers of progenitor cells.

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Dept. Immunobiology
1656 E. Mabel
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Univ. Arizona, Tucson, AZ 85724

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14. ABSTRACT

Experiments were performed exposing mice to varying doses of either JP8 or S8 jet fuel for 1h/day for 7 days, using the new exposure apparatus with in-line monitoring of jet fuel concentrations (aerosol and vapor). Six controls, 6 JP8 exposed and 6 S8 exposed animals examined. There were no significant effect on body weight due to JP8 or S8 exposure, and no effect on immune cell viability from any immune organ due to JP8 or S8 exposure. There was a 53% decrease in Spleen weight due to S8 exposure and a corresponding 40% decrease due to JP8 exposure. There was a 36% decrease in Thymus weight due to S8 exposure and a corresponding 60% decrease due to JP8 exposure. There was a 64% decrease in Thymus cell numbers due to S8 exposure and a corresponding 46% decrease due to JP8 exposure. There was a 20% decrease in Spleen cell numbers due to JP8 exposure only. No significant change in composition in any immune organ due to JP8 or S8 exposure. Both JP8 and S8 exposures using the new exposure chamber resulted in changes in immune function.

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CONCLUSIONS

•JP-8 Exposures at 1000 mg/m^3 for 1h/day for up to 7 days is not overtly toxic

•JP-8 Exposure results in secretion of immunosuppressive cytokines (IL-10, IL-4) by Mac and CD4+ T cells

•Rapid changes in hematology may be a useful biomarker

•Such exposures are inflammatory in nature
–Increase in monocytes, eosinophils, neutrophils and B cells
–Increase in IFNg
–Increases in both apoptosis and necrosis, especially in the thymus
–No CD4+25+ Treg cells were induced by JP-8 exposure

•SP treatment can reverse some but not all JP8 changes

In Vitro JP8 Exposures

1. To elucidate the mechanisms JP8's effect on the immune system (directly or indirectly) immune organs (spleen, thymus and bone marrow) were isolated from naïve animals and placed in culture for periods of 1-7 days. Cells were cultured with various stimuli including media alone, IL-2, Con A +/- IL-2, and stem cell factor. JP-8 jet fuel was added to cultures at either 50 or 100 mg equivalents are per the method of Smulson. Cultures were analyzed at the various timepoints for cell numbers, viability, apoptosis, cytokine secretion, immune cell subsets, and stem/progenitor cell function.
2. In terms of the 50 mg equivalent dose, cells in general required some stimulation to persist past a couple of days in culture. Stimulated cells demonstrated small losses of viability due to JP8 addition (75-80% of control for all organs). No particular immune subset was preferentially sensitive to the effects of jet fuel (although stimulated CD8+ T cells may be slightly more sensitive when stimulated and exposed). CD4+ T cells made more IL-10 when stimulated and JP8 exposed. JP8 did not seem to increase levels of apoptosis induction above that seen with controls. In terms of bone marrow, stem cell function was decreased at d1 following JP8 addition, increased at d4 and then decreased again at d7 (similar to in vivo observations).
3. In terms of the 100 mg equivalent dose, considerable toxicity was observed. Viabilities ranged from 100% at d1 of exposure to 70% at d4 and 45% at d7 for the spleen, while the bone marrow as more resistant being 85% of control even at d7. All cells displayed increased levels of apoptosis even at d1 (as well as d4 and d7). Similar cytokine and stem cell results as in (2) above were observed.

Microarray Analyses

1. In an attempt to identify biological pathways that might reveal mechanisms of JP8's actions on the immune system mice were exposed to 1000 mg/m3 for a single 1h exposure. Organs (spleen, bone marrow and thymus) were isolated at various time points afterwards for RNA isolation and analysis (t=2h, 6h, 12h, 24h and 36h; subsequent time points have also include t=0.5h and 1h).

2. Initially we observed that RNA degradation occurred if the organs were not processed immediately after JP8 exposure, particularly in the t=6h time point. Thus, shorter time points were analyzed to ascertain the cause of the RNA degradation.

3. Analyses of the spleen revealed that 2,515 genes were decreased by JP8 exposure at 2h post-exposure while 917 genes were decreased 3-fold. A total of 1,527 genes were increased at least 2-fold by JP8 exposure at 2h post-exposure, while 403 genes and 86 genes were increased 3-fold and 5-fold respectively.

4. The attached Excel file (Gene Array Summary Overall, please see all tabs) shows examples of genes increased and decreased in the spleen and bone marrow after a single JP8 exposure at 2h and 6h post-exposure.

5. Additional work is being performed to identify pathways induced by jet fuel exposure.

Conclusions

1. JP-8 exposure induces the loss of both 18S and 28S RNA in exposed cells (due to loss of ribosomal proteins) that occurs between 6 and 24 hours after exposure.
2. JP-8 exposure rapidly induces genes associated with a variety of pathways involved in the immune system as well as tumorigenicity.

Year 2006

To elucidate mechanisms of JP8's effect on the immune system (directly or indirectly) immune organs were isolated from naïve animals and placed in culture for periods of 1-7 days with various stimuli. JP-8 was added at either 50 or 100 mg equivalents. Cultures were analyzed at the various timepoints for cell numbers, viability, apoptosis, cytokine secretion, immune cell subsets, and stem/progenitor cell function. The lower dose had minimal effects while the higher dose caused significant toxicity due to apoptosis. To identify biological pathways of JP8's actions on the immune system mice were exposed to 1000 mg/m^3 for 1h. Organs were isolated at timepoints for RNA isolation and analysis. JP-8 exposure induces the loss of both 18S and 28S RNA between 6 and 24 hours after exposure. JP-8 rapidly induces genes associated with pathways involved in the immune system and tumorigenicity. Two experiments were performed

exposing mice to 1000 mg/m^3 JP8 jet fuel for 1h/day for either 1 or 7 days, using the new exposure apparatus with in-line monitoring of jet fuel concentrations (aerosol and vapor). The new exposure apparatus produces aerosol concentrations that are approximately 1/8 that of the previous exposure apparatus. Initial results at the lower "aerosol" concentrations did not reveal as significant effects on the immune system as previous exposures. Additional experiments are in progress to confirm these results. Thus, effects on the immune system may be tied directly to total aerosol JP8 concentration, regardless of total JP8 concentration during exposure.

Years 2007-2008

Experiments were performed exposing mice to varying doses of either JP8 or S8 jet fuel for 1h/day for 7 days, using the new exposure apparatus with in-line monitoring of jet fuel concentrations (aerosol and vapor). The new exposure apparatus produces aerosol concentrations that are approximately 1/8 that of the previous exposure apparatus. That is, a total exposure to 1000 mg/m^3 jet fuel with the new apparatus represents an exposure concentration of approximately 125 mg/m^3 aerosolized JP8 with the old exposure apparatus. Initial results at these lower "aerosol" concentrations did not reveal the obvious outward effects on the immune system as previous exposures with the older exposure chambers (i.e., extreme diminution of spleen and thymus upon isolation). Additional experiments were performed to explore these results.

EFFECTS OF 1000 MG/M^3, 1H/D, 7D EXPOSURES

Six controls, 6 JP8 exposed and 6 S8 exposed animals examined. There were no significant effect on body weight due to JP8 or S8 exposure, and no effect on immune cell Viability from any immune organ due to JP8 or S8 exposure. There was a 53% decrease in Spleen weight due to S8 exposure and a corresponding 40% decrease due to JP8 exposure. There was a 36% decrease in Thymus weight due to S8 exposure and a corresponding 60% decrease due to JP8 exposure. There was no change in Bone marrow cell numbers due to JP8 or S8 exposure. There was a 64% decrease in Thymus cell numbers due to S8 exposure and a corresponding 46% decrease due to JP8 exposure. There was a 20% decrease in Spleen cell numbers due to JP8 exposure only. No significant change in lymphocyte subpopulation composition in any immune organ due to JP8 or S8 exposure.

EFFECTS OF EITHER 4000 OR 8000 MG/M^3, 1H/D, 7D EXPOSURES

(these two levels of exposure are analyzed together as the effects were similar)
Six controls, 6 JP8 exposed and 6 S8 exposed animals for each dose were examined. There was no significant effect on body weight due to JP8 or S8 exposure, and no effect on immune cell Viability from any immune organ due to JP8 or S8 exposure. There was a 20% decrease in Spleen weights due to both JP8 and S8 exposures. There was a 20-40% decrease in Thymus weight due to S8 exposures, and a corresponding 40-50% decrease with JP8 exposures. There was a 50-60% decrease in Spleen cell numbers due to either exposure. There was a 25-45% decrease in Thymus cell numbers due to either exposure. There was a 15-39% decrease in Bone marrow numbers due to either exposure.

For each of these experiments animals have been submitted to University Animal Care for Histopathology analyses. Microarray analyses are being performed to identify pathways involved in the immunotoxicological effects of JP8 exposure.

Thus, both JP8 and S8 exposures using the new exposure chamber result in changes in immune system parameters that warrant further study.

LIST OF MANUSCRIPTS PUBLISHED:

Harris, DT, D. Sakiestewa, D. Titone and M Witten. JP-8 jet fuel exposure induces high levels of IL-10 and PGE2 secretion and is correlated with loss of immune function. Accepted, Tox. Indus. Health, Aug 2007.

Harris, DT, D Sakiestewa, X He, D Titone and M Witten. Effects of in utero JP-8 jet fuel exposure on the immune systems of pregnant and newborn mice. Accepted, Tox. Indus. Health, Aug 2007.

Harris et al, JP-8 Jet Fuel Exposure Potentiates Tumor Development in an Experimental Murine Lung Metastases Model, Toxicology & Industrial Health, Accepted for publication.

Harris et al, JP-8 Exposure Suppresses the Immune Response to Viral Infections, manuscript accepted for publication.